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Evaluation of plant-derived saponins for crude oil dispersion and prospects in environmental remediation

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Abstract

Petroleum contamination continues to threaten soil and water systems in tropical regions, prompting interest in plant-derived saponins as sustainable alternatives to synthetic surfactants for crude oil dispersion. This study evaluated saponins extracted from *Butyrospermum paradoxum* (BP), *Piliostigma reticulatum* (PR), *Waltheria indica* (WI), *Trigonella foenum-graecum* (TFG), and *Jatropha curcas* (JC) to determine their suitability for environmental remediation. Notably, the saponin-rich extracts from these plants have not been previously studied for crude oil dispersion, highlighting the novelty of this work. Saponins were obtained using ultrasound-assisted extraction and purified through a fermentation-based process, yielding extract recoveries ranging from 11.65% (JC) to 32.53% (BP) and total saponin contents between 252.39 mg/g (TFG) and 606.32 mg/g (WI). Purity increased following fermentation, with PR reaching the highest level (70.48%). Interfacial analyses revealed strong variation among species: BP showed the greatest reduction in surface tension (45.41 to 30.43 mN/m), while BP and PR exhibited the lowest critical micelle concentrations (CMC) (60 mg/L). Emulsification stability surpassed the 50% effectiveness threshold in BP (74.67%), WI (64.81%), and PR (57.16%). Droplet size measurements further identified BP (41.43 nm, PDI 0.220) and PR (54.81 nm, PDI 0.226) as capable of forming stable nanoscale emulsions, whereas TFG and JC generated larger, polydisperse droplets with lower stability. Overall, BP and PR emerged as the most efficient natural surfactants, demonstrating strong potential for crude oil dispersion and offering promising prospects for sustainable environmental remediation.

Keywords Saponins, Critical micelle concentration, Emulsification stability, Fermentation-assisted purification, Ultrasound-assisted extraction, Green chemistry

1 Introduction

Oil contamination continues to be an active challenge for ecosystems, and particularly for tropical regions, as oil exploration and leaks affect soil texture, water quality, and biotic diversity [1]. Although traditional remediation methods, such as washing, dispersing, and surfactant-based technologies, have been widely adopted, most man-made



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and petroleum-based surfactants have posed a risk with regard to toxicity, persistence, and secondary pollution [2]. Natural surfactants, including plant-derived saponins, have emerged as promising alternatives because they are biodegradable, environmentally friendly, and capable of reducing interfacial tension, forming stable emulsions, and mobilizing oil at micro and nano-scales. Green surfactants, including L-aspartic acid-based derivatives, have been reported to modify rock wettability, lower interfacial tension, and improve oil recovery in sandstone reservoirs, as demonstrated through microfluidic and core flooding studies [3]. These findings underscore the importance of surface-active properties in the effectiveness of surfactants for petroleum applications. Additionally, findings from mechanistic studies at the oil-water interface, including investigations of surfactant-assisted oil extraction and enhanced oil recovery (EOR), further emphasize that interfacial phenomena such as wettability alteration, interfacial tension reduction, and emulsion stability are fundamental for effective oil mobilization [4].

Saponins, as amphiphilic glycosides with a hydrophobic sapogenin moiety and hydrophilic sugar parts, display strong surfactant properties and thus abilities for surface tension reduction, solubilization, and oil-water emulsion stabilization [5, 6]. Their properties make them applicable for dispersion of crude oil, soil washing, and biostimulation of microorganisms degrading hydrocarbons [7]. Study focus has increasingly shifted toward saponins from renewable plant materials as biologically stable and economically advantageous natural substitutes for dispersants [8–10]. Also, saponins from plants have been shown capable of producing nanoemulsions with oil, thus enhancing interactions with microorganisms and improvements in biodegradation rates [11]. Despite these merits, marked variability following diversity in structure and interfacial properties depending on oil type and composition have been noticed [12, 13].

Aside from cleansing and rehabilitating the environment, natural surfactants and biosurfactants, particularly saponins, have growing uses in petroleum [14]. These substances have several uses in the EOR process because they can improve oil mobility and alter the wettability of rocks. Moreover, they can also reduce interfacial tension and mitigate clay and shale swelling caused by drilling and water injection [15–17]. These uses emphasize the importance of understanding interfacial properties associated with saponins extracted from plants.

In this context, a systematic comparative assessment of plant-derived saponins is needed to identify environmentally benign dispersants with strong surface activity, low critical micelle concentrations, and high emulsion stability [18]. Accordingly, saponins were extracted from selected underexplored tropical plants (*Piliostigma reticulatum* (PR), *Butyrospermum paradoxum* (BP), *Trigonella foenum-graecum* (TFG), *Jatropha curcas* (JC), and *Waltheria indica* (WI)) chosen for their ethnobotanical relevance, tropical distribution, and reported saponin richness. Key interfacial and dispersion-related properties, including surface tension reduction, critical micelle concentration, droplet size distribution, and polydispersity index, were systematically evaluated to establish links between physicochemical characteristics and dispersion efficiency. Notably, these saponin-rich extracts have not previously been investigated for crude oil dispersion, making this the first comparative, data-driven study to relate fermentation-purified saponin properties to dispersion performance. The findings highlight the potential of these tropical plant saponins as eco-friendly dispersants for oil remediation and petroleum-related

Table 1 Saponin-Rich plants utilized in the study

Scientific names	English common names	Hausa names	Parts used
<i>Butyrospermum paradoxum</i>	Shea butter	Kadanya	Leaves
<i>Waltheria indica</i>	Sleepy morning	Hankufa	Leaves
<i>Piliostigma reticulatum</i>	Camel's foot	Kargo/Kalgo	Leaves
<i>Jatropha curcas</i> L.	Physic nut	Bini da zugu	Stem-bark
<i>Trigonella faenum graecum</i>	Fenugreek	Hulba	Seeds



Shea Butter Leaves



Fenugreek Seeds



Sleepy Morning Leaves



Camel's Foot Leaves



Physic Nut Stem-Bark

Fig. 1 Pictorial overview of the plants employed for saponin extraction

applications such as enhanced oil recovery and clay inhibition, supporting the development of more sustainable technologies in biodiverse, oil-producing regions.

2 Methodology

2.1 Collection and identification of plant samples

Plant materials were collected from various locations across Kano State, Nigeria. Leaves of Shea butter (*Butyrospermum paradoxum*) were obtained from Galija in Madobi (11.7444° N, 8.3851° E) and Kura Local Government Areas. Sleepy morning (*Waltheria indica*) leaves were collected from Rantan in Bebeji (11.6085° N, 8.2089° E) and Wangara in Tofa (11.9108° N, 8.4146° E). Camel's foot (*Piliostigma reticulatum*) leaves were sourced from Kwanar Dawaki in Dawakin Kudu (11.8347° N, 8.5981° E), while stem bark of Physic nut (*Jatropha curcas* L.) was collected from Dan Hassan in Kura (11.7531° N, 8.3457° E). Fenugreek (*Trigonella foenum-graecum*) seeds were procured from the International Institute of Tropical Agriculture (IITA) station in Tarauni, Kano (11°58'49" N, 8°33'26.5" E; altitude 492.5 m). Their taxonomic identification and authentication were carried out by Dr. Yusuf Nuhu, a botanist from the Department of Plant Science, Bayero University, Kano. The plant species used in this research are listed in Table 1.

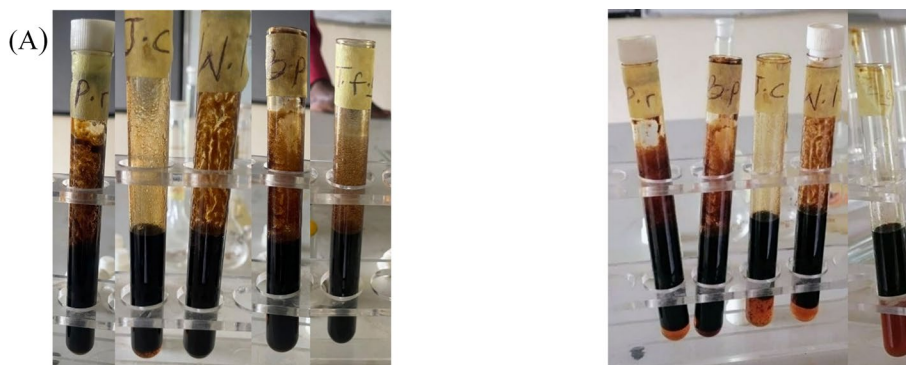


Fig. 2 Emulsification test illustration for E24 determination; **A**-immediately after mixing and **B**-after 24 h

2.2 Preparation of plant material

Leaves, fruits, seeds, and stem bark from the saponin-rich plants were carefully separated from their stalks, rinsed with clean water, and air-dried. The dried materials were pulverized into fine powder using a mechanical grinder, sieved through a 0.5 mm mesh, and stored in airtight containers [19]. To remove any residual moisture, the powdered samples were further oven-dried at 40 °C for one hour before extraction [8].

2.3 Saponin extraction procedure

Finely ground plant powders (leaves, seeds, or stem bark) were treated with 70% aqueous ethanol at a solid-to-solvent ratio of 1:15 (w/v) for 20 min, with the process repeated three times. Ultrasonication was carried out at a frequency of 40 kHz and maintained at 20 °C. The extracts were centrifuged at $3400 \times g$ for 10 min, after each extraction cycle, the supernatant was collected, and all three supernatants were pooled before concentration under reduced pressure at 50 °C using a rotary evaporator [20, 21]. The extraction yield was calculated cumulatively based on the total dried mass obtained from the pooled extracts.

The extraction yield (EY) was then calculated and expressed according to Eq. 1.

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of Extract}}{\text{Weight of Plant Material}} \times 100 \quad (1)$$

2.4 Fermentation-based saponin purification

This method is selected because fermentation-assisted purification enhances saponin extraction by using microbial or enzymatic activity to release saponins from plant matrices, remove impurities, and preserve bioactivity, offering a milder and more environmentally friendly alternative to conventional solvent-based methods.

2.4.1 Yeast-assisted crude saponin extract fermentation

2.4.1.1 Activation of yeast Before inoculation, 3 g of dry *Saccharomyces cerevisiae* yeast was rehydrated in a mixture containing 15 mL of distilled water and 15 mL of crude saponin extract. The suspension was activated in a water bath at 35 °C for 30 min and subsequently used for fermentation [22].

2.4.1.2 Fermentation process The activated yeast culture was inoculated into 150 mL of crude saponin extract obtained from the extraction process. The mixtures were sealed

and incubated at ambient temperature (25–35 °C), protected from direct sunlight, for 96 h (4 days). After fermentation, samples were collected and centrifuged at 8000 rpm for 10 min to separate yeast cells [22, 23]. The resulting supernatant was then analyzed to determine:

- (i) Total saponins according to the Vanillin-Sulfuric acid assay [24].
- (ii) Content of soluble solids.
- (iii) Purity % = $\frac{W_i}{W_s} \times 100$

Here, purity (%) denotes the percentage of total saponins, W_i (g) is the mass of total saponins in the extract, and W_s (g) represents the soluble solids content [25].

2.5 Qualitative saponin test

A 2.5 mL aliquot of the extract was mixed with a few drops of distilled water and shaken vigorously. The formation of a stable and abundant froth confirmed the presence of saponins [26].

2.6 Determination of total saponin content

The total saponin content (TSC) of the plant extracts was assessed using the vanillin–sulfuric acid colorimetric method, following the procedure of Du MengHao and co-workers [27] with minor modifications. In brief, 1.0 mL of the fermented plant extract was placed into a 10 mL stoppered test tube and combined with 1.0 mL of 8% (w/v) vanillin in ethanol. The tubes were kept in an ice-water bath, after which 8.0 mL of 72% (v/v) sulfuric acid was added gradually with gentle mixing. The mixtures were incubated at 60 °C for 30 min in a water bath, then cooled in an ice-water bath for 10 min and allowed to equilibrate to room temperature. Absorbance was measured at 560 nm using a UV–VIS spectrophotometer [27].

A commercial saponin standard at varying concentrations was used to generate a calibration curve. The saponin concentration in the extracts was calculated from the linearized calibration equation. The total saponin content (mg/g) was then determined based on the total saponin amount relative to the initial mass of powdered extract, as described in Eq. 2 [28].

$$\text{Total Saponin Content (mg/g)} = \frac{\text{Total saponin amount (mg)}}{\text{Initial mass of powdered extract (g)}} \quad (2)$$

2.7 Preliminary evaluation of saponin-rich extracts

2.7.1 Solubility assessment

The saponin-rich extracts were first dried in a desiccator over calcium oxide (CaO). Their solubility in water, methanol, ethanol, chloroform, petroleum ether, dilute NaOH, and dilute HCl was evaluated as follows: 1 mg of each dried extract was added to 1 mL of the respective solvent and stirred until saturation at room temperature. Saturation was confirmed by the presence of undissolved residues. The solubility in each solvent was then qualitatively assessed through visual observation [29].

2.7.2 Surface activity and determination of critical micelle concentration (CMC)

Surface tension was measured using the capillary rise method solely for the purpose of determining the CMC, following standard practice in saponin and biosurfactant studies

where CMC is conventionally derived from air-liquid surface tension trends rather than liquid-liquid interfacial tension [30]. Solutions of the plant extracts were prepared at concentrations of 20, 50, 100, 200, and 400 mg/L, and all surface tension measurements were carried out at 25 ± 1 °C. The setup was calibrated using distilled water to confirm a reference surface tension value of 72.0 mN/m. Capillary tubes, each labeled uniquely, were thoroughly cleaned and fully wetted inside and out to avoid errors from trapped air or incomplete wetting. All readings were taken at eye level to eliminate parallax error, and each measurement was performed in triplicate. An optical pin was positioned so that its tip just contacted the liquid surface, serving as a reference baseline. The microscope was aligned so that its horizontal cross-wire was tangential to the pin tip, and the reading was recorded.

Each wetted capillary tube was then carefully inserted into the extract solution, ensuring no droplets remained on the surface. The meniscus of the liquid rising within the tube was focused through the microscope, tangential to the horizontal cross-wire, and the reading was recorded. The capillary rise height, h , was calculated by subtracting the baseline reading from the meniscus reading. This process was repeated for all tubes at each extract concentration.

The internal radius r of each capillary tube was determined by measuring the internal diameter at two perpendicular orientations using the travelling or vernier microscope, averaging the values, and dividing by two. Surface tension, T , was calculated using the capillary rise Eq. 3:

$$T = \frac{\rho g h r}{2} \quad (3)$$

where ρ is the liquid density at the recorded temperature, g is the standard acceleration due to gravity, h is the capillary rise, and r is the internal radius of the tube. Surface tension values were plotted against concentration, and CMC was determined from the intersection of the linear segments on the surface tension versus concentration graph [31].

2.7.3 Emulsion stability assessment

The emulsification index was determined following the methods of [32, 33]. Briefly, 3.0 mL of a hydrophobic substance (crude oil) was mixed with 3.0 mL of each saponin extract (each extract was prepared in distilled water; aqueous phase pH = 7.0, salinity = 0.0 gL^{-1}) and samples were homogenized using a vortex mixer (infitek VMX3-28) at 1200 rpm for 1 min in 15 mL tubes (internal diameter 1.5 cm). After 24 h, the emulsion index (E24) was calculated using Eq. 4:

$$E_{24} = E/H \times 100 \quad (4)$$

where E is the height of the emulsion layer and H is the total height of the mixture, both measured in centimeters. All tests were performed in triplicate.

2.7.3.1 Characterization of the oleic phase The oleic phase used for all emulsification and surface activity experiments consisted of crude oil sourced from the Niger Delta Western Field, Warri, Delta State, Nigeria. The crude oil had a density of 0.964 g/cm^3 at ambient laboratory temperature, the viscosity was 12 cP and an API gravity of 15.1°, clas-

sifying it as a heavy crude. The sample was used as collected, without any pre-treatment or compositional modification. SARA fractionation (saturates, aromatics, resins, and asphaltenes) and viscosity measurements were not determined in the present study.

2.7.4 Droplet size and polydispersity analysis

The average droplet size of the prepared water-in-oil (W/O) emulsions was determined using Dynamic Light Scattering (DLS). Each sample was measured at least six times, and the mean values for droplet diameter and polydispersity index (PDI) were calculated. Measurements were conducted at 25 °C, approximating room temperature, with an equilibration time of 3 min for the first reading. The DLS system used automatic laser beam regulation ($\lambda = 658$ nm), and the optical settings were adjusted individually for each measurement. Autocorrelation functions of the backscattered light were recorded at a scattering angle of 175° [34]. DLS was used to provide quantitative insights into droplet/aggregate size and polydispersity within the crude-oil/saponin emulsions. This complements the emulsification index measurements by providing nanoscale resolution of dispersion behavior.

3 Results and discussion

3.1 Comparative yield of saponin extracts

Ultrasound-assisted extraction (UAE) played a central role in enhancing recovery by promoting cell rupture and solvent diffusion, outperforming conventional techniques in both yield and preservation of bioactivity. Previous studies by numerous researchers have shown the efficiency of UAE techniques in enhancing solvent diffusion [35, 36]. Notably, the UAE yields of the plant extracts varied considerably, with BP showing the highest yield of 32.53% (Fig. 3). The yield obtained for WI, PR, TFG and JC were 27.88%, 23.48%, 21.97% and 11.65% respectively.

The higher yields in BP and WI suggest that their softer tissue matrices facilitated cavitation disruption and solvent penetration, consistent with reports that UAE improves saponin recovery in plants with more accessible cellular structures [37–39]. By contrast,

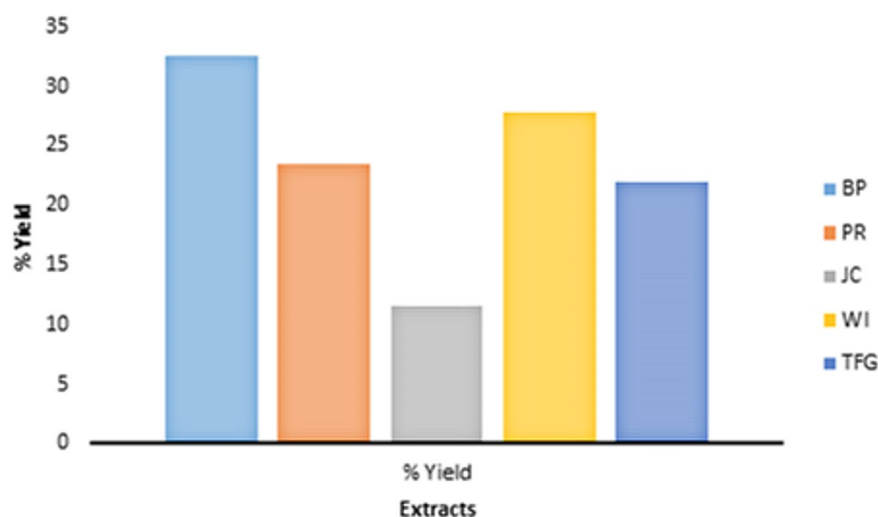


Fig. 3 Extraction yield of the saponin-rich extracts. Values represent single extraction determinations; error bars are not shown

the low yield in JC may be due to its denser and lignified tissues, which restrict solubilization, a trend previously reported for *Jatropha species* in similar extraction studies [29, 40].

The use of aqueous ethanol was also effective, aligning with previous findings that 50–80% ethanol optimally dissolves both hydrophilic and moderately lipophilic saponins [41]. Although yield alone does not determine performance in crude oil dispersion, the relatively high recoveries of BP and WI provide a practical advantage for producing sufficient biosurfactants for environmental remediation applications [42].

3.2 Frothing test for saponin presence

The frothing test confirmed the presence of saponins in all extracts studied, although the intensity of foam formation varied across species (Table 2). TFG exhibited the strongest froth (+++), indicating a higher concentration of surface-active saponins, while BP and JC produced moderate froths (++), PR and WI showed only weak foam formation (+). The persistence and thickness of the froth is a qualitative indication of saponin abundance, since these amphiphilic glycosides stabilize air-water interfaces through micelle formation [43, 44].

The variation observed among species reflects differences in saponin content and structural composition. TFG's strong froth aligns with earlier reports that fenugreek seeds are rich in steroidal saponins with pronounced foaming properties [45, 46]. By contrast, the weak froths observed in PR and WI suggest lower levels or less surface-active saponin types, a pattern also noted in legumes and shrubs where triterpenoid saponins are bound within complex cell matrices [47]. The moderate froth in JC is consistent with findings that *Jatropha curcas* contains appreciable but variable amounts of saponins depending on plant part and extraction conditions [29].

From an application perspective, the frothing ability of extracts provides a preliminary measure of their surfactant potential for crude oil dispersion. Extracts like TFG and BP, which demonstrated stronger foaming, are likely to exhibit better emulsification and interfacial activity, traits directly relevant to hydrocarbon mobilization in contaminated environments [48, 49].

3.3 Total saponin content of the extracts

The total saponin content of the extracts, determined using the vanillin–sulfuric acid colorimetric assay, varied significantly among the studied plants (Table 3). WI exhibited the highest concentration (606.32 ± 0.99 mg/g), followed by BP, (462.09 ± 1.10 mg/g). Moderate levels were recorded in PR, (299.20 ± 0.64 mg/g) and JC, (287.97 ± 0.64 mg/g), while TFG, (252.39 ± 2.08 mg/g) showed the lowest content. The statistical differences

Table 2 Qualitative test for saponins

Samples	Froth formation	Froth level
BP	Y	+++
TFG	Y	+++
JC	Y	++
PR	Y	+
WI	Y	+

KEY: 'Y' indicates the presence of froth. '+++ ' indicates a strong froth formation. '++ ' indicates a moderate froth formation. '+' indicates a weak froth formation

Table 3 Total saponin content of the extracts studied (mg/g)

Sample	Total saponin content $\pm$ SEM
BP	462.09 $\pm$ 1.10 ^{ab}
JC	287.97 $\pm$ 0.64 ^a
TFG	252.39 $\pm$ 2.08 ^a
WI	606.32 $\pm$ 0.99 ^b
PR	299.20 $\pm$ 0.64 ^a

KEY: values are mean $\pm$ standard error of mean (SEM) from replicate measurements ($n = 3$). Mean with different superscript alphabet in a column differed significantly ($p < 0.05$)

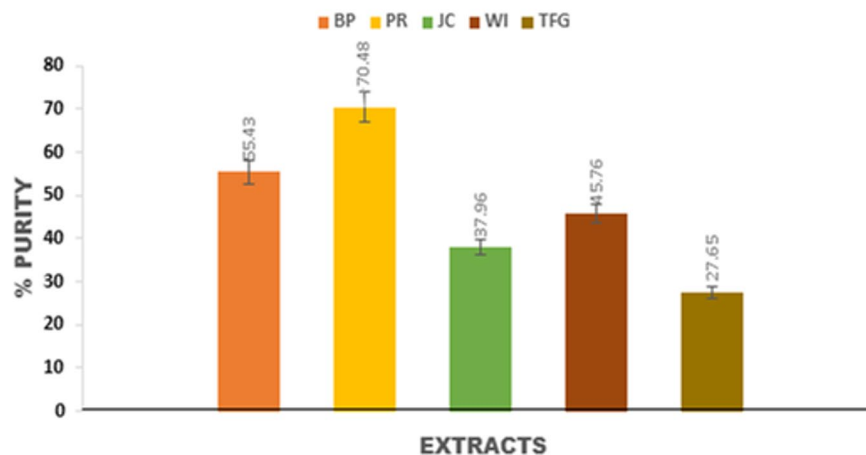


Fig. 4 Purity of the fermented purified saponin-rich extracts. Values are represented as mean $\pm$ standard deviation (SD) from replicate measurements ($n = 3$)

($p < 0.05$) highlight species-specific variations in saponin biosynthesis, tissue distribution, and extractability under UAE conditions.

The high concentration observed in WI underscores its potential as a rich source of triterpenoid saponins, consistent with recent findings on *Malvaceae* species that reported similar elevated levels using vanillin-sulfuric acid quantification [50]. BP also demonstrated substantial saponin levels, aligning with studies that identified Shea tree tissues as reservoirs of bioactive saponins [51]. By contrast, the relatively lower concentrations in TFG and JC are in agreement with earlier reports documenting moderate saponin accumulation in fenugreek seeds and variable yields in physic nut depending on tissue type and growth stage [52, 53].

From a functional standpoint, extracts with higher total saponin content are more likely to demonstrate efficient surfactant behavior, including reduction of interfacial tension, micelle formation, and emulsification of hydrophobic pollutants. This is particularly relevant for crude oil dispersion, where higher saponin levels may translate into lower application doses for effective remediation [54].

3.4 Purity of the fermented purified extracts

The purity of saponin extracts following microbial fermentation showed marked differences among the studied species (Fig. 4). PR displayed the highest purity (70.48%), followed by BP, 55.43% and WI, 45.76%, while JC, 37.96% and TFG, 27.65% exhibited lower values. The significant enrichment observed in PR demonstrates that fermentation effectively removed interfering metabolites such as polysaccharides, proteins, and phenolics,

thereby increasing the relative saponin content. This agrees with recent studies highlighting the ability of microbial enzymes to selectively degrade non-saponin matrices, leading to improved purity of phytochemical extracts [55].

The comparatively high purity obtained in BP and WI also suggests that their tissue composition is favorable for microbial action, enabling efficient breakdown of co-extracted compounds. The relatively low enrichment in TFG and JC may be attributed to the presence of tightly bound saponin-protein or saponin-polyphenol complexes that resist microbial degradation, a limitation previously reported in legumes and *Jatropha* species [29, 56].

From an environmental application perspective, higher saponin purity is directly advantageous for crude oil dispersion, as purer extracts generally exhibit lower critical micelle concentration, stronger interfacial tension reduction, and more stable emulsification capacity [57, 58]. Moreover, the adoption of fermentation as a purification strategy is particularly relevant for environmental remediation since it is cost-effective, eco-friendly, and aligns with green chemistry principles [59].

3.5 Emulsification stability of saponin-rich extracts

The emulsion stability test revealed distinct differences among the plant extracts (Table 4). BP exhibited the highest stability ($74.67 \pm 1.78\%$), followed closely by WI, ($64.81 \pm 0.90\%$) and PR, ($57.16 \pm 2.11\%$). These three extracts demonstrated moderate to strong emulsification potential, significantly higher than that observed in TFG, ($26.19 \pm 1.89\%$) and JC, ($23.26 \pm 4.60\%$), which showed comparatively weak stability.

The superior performance of BP, WI, and PR suggests that these extracts contain higher proportions of surface-active saponins capable of effectively lowering interfacial tension and stabilizing oil-water emulsions. This agrees with earlier reports that emphasized the critical role of saponin structure particularly the balance between hydrophilic sugar moieties and hydrophobic aglycones in stabilizing emulsions [60, 61]. Moreover, similar trends have been observed for other plant-based green surfactants applied in petroleum systems; for example, *Acanthophyllum* root extract demonstrated that emulsion stability and interfacial tension reduction are key determinants of oil mobilization under varying ionic conditions in carbonate reservoirs [18].

From a structure-function perspective, the enhanced emulsification stability observed for BP and PR can be rationalized by the dominant saponin chemotypes present in these plants and their resulting hydrophilic-lipophilic balance (HLB) [62]. Although detailed structural elucidation of individual saponins from the leaves of BP and PR has not yet been reported, related compounds have been characterized from their stem bark. BP is generally enriched in triterpenoid saponins, particularly butyrospermol-derived aglycones, while PR predominantly contains oleanane-type triterpenoid glycosides. These

Table 4 Emulsification stability (%) of Saponin- rich extracts from various plant sources

Sample	% emulsification stability $\pm$ SEM
BP	74.67 ± 1.78^{ab}
WI	64.81 ± 0.90^{ab}
PR	57.16 ± 2.11^{ab}
TFG	26.19 ± 1.89^b
JC	23.26 ± 4.60^b

KEY: values are mean $\pm$ Standard Error of Mean (SEM) from replicate measurements ($n = 3$). Mean with different superscript alphabet in a column differed significantly ($p < 0.05$)

saponins typically feature a bulky hydrophobic triterpenoid backbone coupled with multiple hydrophilic sugar moieties [63], a structural arrangement that confers an optimal HLB for interfacial activity. The presence of extended or branched oligosaccharide chains, especially in PR, increases the hydrophilic volume and promotes steric stabilization by thickening the interfacial layer, thereby limiting droplet-droplet interactions [64]. However, the rigid triterpenoid aglycone in BP acts as a strong hydrophobic anchor, enhancing adsorption at the oil-water interface and contributing to the formation of cohesive interfacial films. Collectively, these structural attributes favor dense molecular packing at the interface, suppress coalescence, and yield superior emulsion stability. Conversely, the poor stability observed in TFG and JC may reflect either lower concentrations of amphiphilic saponins or the presence of co-extracted components that interfere with micelle formation, as previously noted in some studies [21, 65].

From an application standpoint, emulsion stability above 50% is generally considered effective for crude oil dispersion in contaminated environments as reported by Li and co-workers [66]. The ability of BP, WI, and PR to form stable emulsions not only enhances the solubilization and bioavailability of hydrophobic pollutants but also supports microbial degradation processes, aligning with recent findings that highlight the synergy between natural surfactants and microbial consortia in oil remediation systems [67–69].

3.6 Surface tension of saponin-rich extracts

The measurement of surface tension across increasing concentrations of the extracts (20–400 mg/L) revealed notable differences in their interfacial activity (Table 5; Fig. 5). BP consistently demonstrated the strongest surface-active potential, with values reducing sharply from 45.41 mN/m at 20 mg/L to 30.43 mN/m at 400 mg/L. This places BP as the most efficient extract in lowering surface tension, achieving values comparable to those reported for effective natural surfactants [70, 71]. Similarly, JC showed strong surface activity, decreasing from 53.77 mN/m at 20 mg/L to 34.33 mN/m at 400 mg/L, suggesting the presence of highly amphiphilic saponins capable of reducing interfacial energy.

By contrast, PR and WI demonstrated moderate reductions in surface tension, reaching 43.91 and 50.36 mN/m at 400 mg/L, respectively. Their relatively higher residual surface tension values suggest either lower concentrations of triterpenoid saponins or differences in molecular structures that influence micelle formation efficiency [72, 73]. TFG, however, exhibited the weakest effect, with surface tension remaining above 52 mN/m even at 400 mg/L, indicating limited surfactant potential compared to the other extracts. This may be due to a relatively low saponin concentration or a unique molecular

Table 5 Calculated surface tension (mN/m) of Saponin-Rich extracts studied

Samples	Concentrations mg/L				
	20	50	100	200	400
PR	60.57 ± 0.68 ^{aE}	51.25 ± 0.46 ^{aD}	48.81 ± 0.73 ^{aC}	46.24 ± 0.68 ^{aB}	43.91 ± 0.59 ^{aA}
WI	72.56 ± 0.85 ^{aE}	58.63 ± 0.79 ^{aD}	54.92 ± 0.68 ^{aC}	51.98 ± 0.62 ^{aB}	50.36 ± 0.57 ^{aA}
BP	45.41 ± 0.77 ^{aD}	35.61 ± 0.71 ^{aC}	33.21 ± 0.67 ^{aB}	32.36 ± 0.64 ^{aA}	30.43 ± 0.58 ^{aA}
JC	53.77 ± 0.84 ^{aE}	41.66 ± 0.73 ^{aD}	38.80 ± 0.68 ^{aC}	35.44 ± 0.61 ^{aB}	34.33 ± 0.55 ^{aA}
TFG	71.71 ± 0.99 ^{aA}	59.90 ± 1.09 ^{aB}	57.90 ± 0.56 ^{aB}	54.10 ± 0.59 ^{aB}	52.61 ± 0.56 ^{aB}

Key: Values are mean ± SD ($n = 3$). Different lowercase superscript letters in the same column indicate significant differences among extracts at the same concentration ($p < 0.05$). Different uppercase superscript letters in the same row indicate significant differences across concentration for same extract ($p < 0.05$)

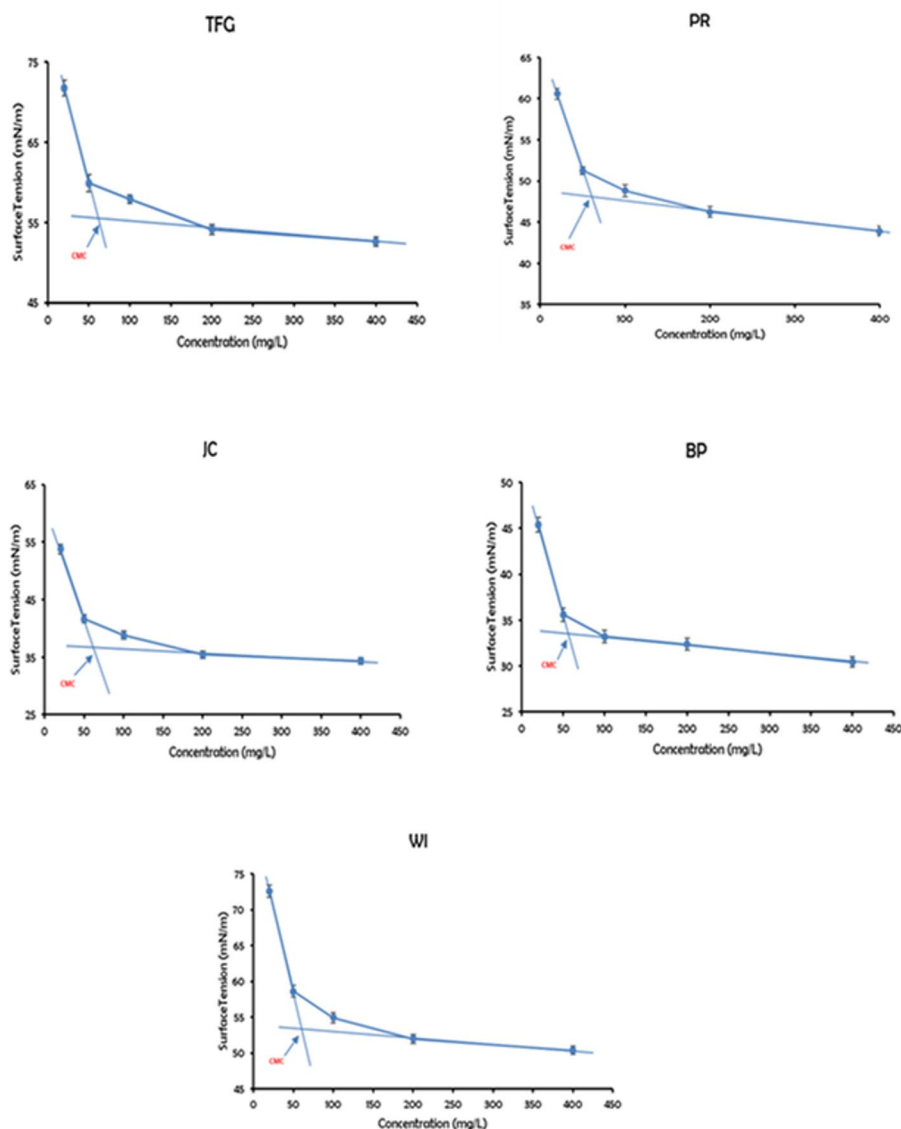


Fig. 5 Plots of Surface Tension vs. Concentration Showing the CMC of The Extracts. Error bars represent mean $\pm$ standard deviation (SD) of replicate measurements ($n=3$)

structure that could influence its functional activity. The concentration- dependent decline in surface tension across all extracts highlights the amphiphilic nature of plant-derived saponins, consistent with previous findings that increased concentrations enhanced micellization and interfacial adsorption [57, 74].

3.7 Critical micelle concentration (CMC) of saponin-rich extracts

The CMC values derived from the surface tension concentration plots ranged from 60 to 92 mg/L, indicating clear differences in surfactant efficiency among the extracts (Fig. 6). BP and PR both exhibited the lowest CMC values (60 mg/L), suggesting they form micelles more readily at lower concentrations and are thus the most surface - active extracts. This behavior highlights their strong amphiphilic nature and agrees with recent findings that lower CMC values are characteristic of efficient biosurfactants capable of effective interfacial adsorption and crude oil solubilization [75, 76].

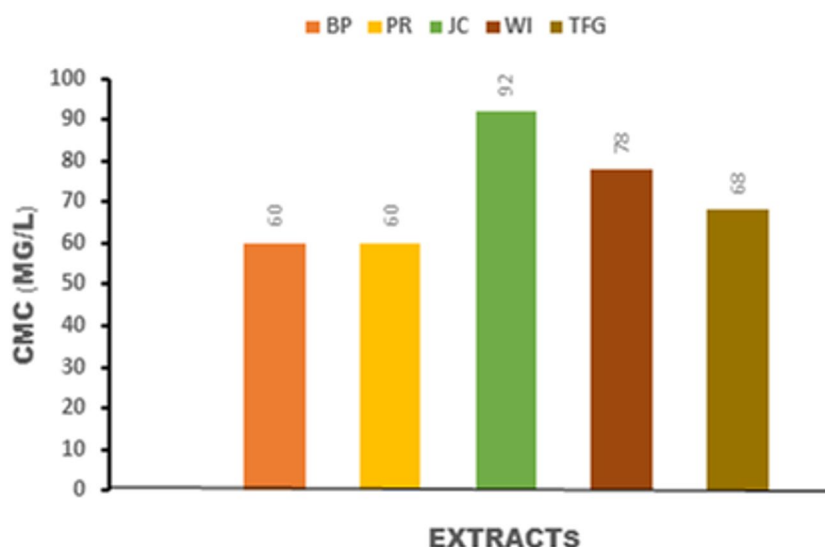


Fig. 6 Critical Micelle Concentration values of the extracted saponins determined by surface tension measurements

WI and TFG demonstrated intermediate CMC values of 78 mg/L and 68 mg/L, respectively. Although higher than BP and PR, these values still fall within the effective range reported for natural surfactants. For instance, Bustos and colleagues reported CMC values of ~ 1200 mg/L for quinoa-derived saponins [56], while Wang and co-workers observed functional micellization of soapnut saponins at comparably high concentrations [72]. Thus, the moderate values of WI and TFG suggest they remain effective bio-surfactants compared to other plant-derived systems described in recent literatures [58, 77].

In contrast, JC showed the highest CMC value (92 mg/L), reflecting a weaker tendency for micellization and suggesting a higher concentration is required to achieve effective aggregation, which is consistent with its relatively lower surface tension reduction performance. The observed variation highlights the influence of saponin structure and distribution across different plant species. Extracts with lower CMC values, particularly BP and PR, stand out as more efficient candidates for environmental remediation, since they require lower doses to achieve micelle formation and enhance crude oil dispersion. This aligns with recent studies stressing the importance of low CMC saponins and other natural surfactants in cost-effective and sustainable bioremediation strategies [78–80].

Existing literature showed that Pure Quillaja saponin typically exhibits surface tension values in the range of 28–35 mN/m at concentrations between 200 and 500 mg/L, with CMC values reported between 50 and 120 mg/L depending on purity and source [81]. Similarly, commercial soapnut saponin has been shown to reduce surface tension to approximately 33–38 mN/m and demonstrates CMC values ranging from 70 to 150 mg/L in aqueous systems [9]. When compared to these benchmarks, the BP and PR extracts in this study display competitive performance: BP achieved a surface tension of 30.43 mN/m at 400 mg/L and a CMC of 60 mg/L, while PR reached 43.91 mN/m with the same CMC value. These results indicate that BP, in particular, performs comparably to some commercial saponins, suggesting high purity or the presence of structurally potent triterpenoid saponins. PR, although less efficient in lowering surface tension,

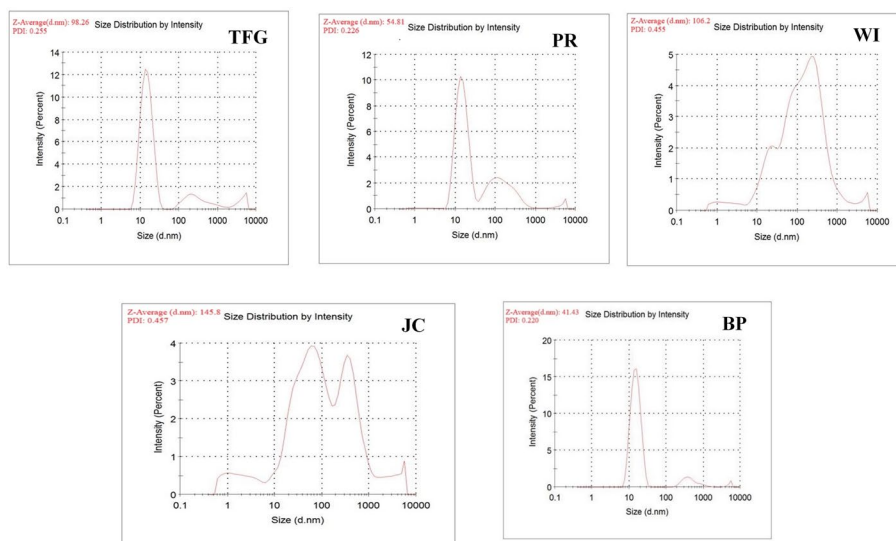


Fig. 7 Intensity-Based Droplet size Distribution Curves of The Extracts (TFG; PR; WI; JC and BP): Showing the intensity-weighted distribution of droplet sizes, indicating the relative abundance of different droplet sizes in each extract

still falls within the functional range of several natural and commercial saponin systems reported in literature.

3.8 Dynamic light scattering analysis (DLS) of saponin-rich extracts

DLS analysis revealed distinct differences in the emulsification efficiency of the saponin-rich extracts (Fig. 7). BP (41.43 nm, PDI 0.220) produced the smallest droplet size with a sharp unimodal distribution, confirming its superior emulsification and nanoemulsion stability. Such nanoscale droplets with low PDI values (< 0.3) are consistent with reports that biosurfactant-based nanoemulsions below 50 nm exhibit enhanced thermodynamic stability, reduced Ostwald ripening, and effective interfacial activity for crude oil dispersion [82, 83].

PR (54.81 nm, PDI 0.226) also generated nanoscale droplets with a narrow unimodal peak, demonstrating good uniformity and stability. Its performance is comparable to optimized saponin emulsions reported in literature, where droplet sizes below 100 nm with PDIs under 0.25 confer resistance to coalescence and long-term stability [84, 85]. Notably, TFG (98.26 nm, PDI 0.255) produced moderately uniform nanoemulsions with a unimodal distribution, though slightly larger in size compared to BP and PR. The PDI remains below the 0.3 threshold, indicating acceptable stability. Fenugreek's amphiphilic steroidal saponins and hydrocolloids such as galactomannan are known to enhance emulsification efficiency, aligning with previous reports of its emulsifying and stabilizing capacity [86, 87].

By contrast, WI (106.2 nm, PDI 0.455) and JC (145.8 nm, PDI 0.457) exhibited significantly broader and multimodal droplet size distributions, reflecting heterogeneous emulsions prone to coalescence. PDI values above 0.4 are generally considered highly polydisperse and unstable, leading to limited kinetic stability [88, 89]. Such broad distributions are often associated with incomplete surface coverage when surfactant concentration falls below the CMC, or when structurally diverse saponins lead to inconsistent interfacial adsorption [90, 91].

4 Limitations and future work

Although this study provides valuable insight into the interfacial behavior of saponin-rich plant extracts, several limitations should be acknowledged. First, the extracts were partially purified rather than isolated as individual saponins; consequently, advanced structural characterization techniques such as FTIR, NMR, Raman spectroscopy, LC-MS, and TGA were not applied. Future studies will employ these tools to elucidate molecular structures and establish clearer structure-function relationships.

The CMC was determined solely from surface tension measurements due to the unavailability of complementary techniques such as interfacial tension, electrical conductivity, and turbidity-based analyses at the time of experimentation. Incorporating these methods in future work would strengthen CMC determination and interpretation.

All experiments were conducted under controlled baseline conditions using distilled water (pH 7.0, zero salinity). Given that salinity level and ionic composition significantly influence micellization behavior, emulsion stability, and surfactant performance in petroleum systems, systematic evaluation under varying salt types and concentrations is necessary for practical applications in marine and reservoir environments.

In addition, only surface tension measurements were performed, while direct oil-water interfacial tension was not evaluated. Future studies should incorporate pendant-drop or spinning-drop techniques to more accurately assess oil-water interfacial phenomena relevant to crude oil dispersion.

Finally, the work was limited to laboratory-scale extraction and evaluation of five tropical plant species. The performance of these saponins under field conditions, across different crude oil types, as well as large-scale production feasibility, process optimization, cost analysis, and long-term stability remain important areas for future investigation.

5 Conclusion

This study demonstrated that the specific tropical plant-derived saponins evaluated, differed markedly in interfacial and emulsification performance relevant to crude oil dispersion. The study aimed to comparatively assess the surface activity, emulsification stability, and micellar characteristics of saponin-rich extracts from BP, PR, WI, TFG, and JC, and this objective was achieved through systematic physicochemical and functional analyses. Among the extracts tested, BP and PR consistently exhibited superior performance, characterized by lower CMC (60 mg/L), higher emulsification stability (BP: 74.67%; PR: 57.16%), greater surface tension reduction (BP: 74.67%; PR: 57.16%), and the formation of nanoscale oil droplets with relatively narrow PDI values (BP: 41.43 nm, PDI 0.220; PR: 54.81 nm, PDI 0.226). WI showed moderate effectiveness, likely associated with its relatively high saponin content, while TFG and JC were less effective, particularly in sustaining stable emulsions over time. These findings indicate that saponin effectiveness is plant-specific and governed more by functional surface properties than by extraction yield or total saponin content alone.

This study provides a solid comparative, data-driven evaluation of fermentation-purified saponins from selected tropical plants for crude oil dispersion, linking functional properties including surface tension reduction, CMC, emulsification stability, droplet size, and polydispersity to performance. The environmentally friendly ultrasound-assisted extraction and fermentation-based purification methods enhance

reproducibility, sustainability, and standardization across plant species, offering a robust framework for future bioremediation studies.

Overall, the results identify BP and PR extracts as the most promising candidates among the plants studied for further investigation as natural surfactants in crude oil dispersion systems. While the study was limited to laboratory-scale extraction and evaluation, it provides a physicochemical basis for selecting plant saponins with favorable surface activity for subsequent soil microcosm or field-oriented bioremediation studies. Further work is required to optimize extraction processes, evaluate cost-effectiveness, and validate performance under realistic environmental conditions before large-scale application can be considered. From a practical perspective, the scalability and economic feasibility of the fermentation-based purification method employed in this study are important considerations for real-world oil spill remediation. Notably, the fermentation-assisted approach operates under mild conditions and low solvent demand, suggesting potential suitability for scale-up. Nevertheless, further investigations into process optimization, cost modeling, and industrial-scale extraction performance are necessary to fully assess its viability for large-scale crude oil remediation.

Author contributions

FMM : Investigation, Methodology, Data Curation, Formal Analysis, Writing of Original Draft; AAA : Supervision, Conceptualization, Methodology, review and editing of original manuscript; SCJ: Resources, Validation, Visualization and editing of original manuscript HTB: Software, Data Analysis, Visualization, and editing of original manuscript; AMM: Investigation, and editing of original manuscript; AAG: Validation and editing of original manuscript. All authors have read and approved the final version of the manuscript.

Funding

This research was supported by the Petroleum Technology Development Fund (PTDF) under Reference Number PTDF/ED/ISS/PHD/FMM/2055/21.

Data availability

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 1 October 2025 / Accepted: 11 February 2026

Published online: 16 February 2026

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